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Metal Complexes of Anti-inflammatory Drugs. Part VI. 2-Aminomethyl-4(1,1-dimethylethyl)-6-iodophenol (MK-447) Complex of Copper(II)

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Abstract

The preparation and properties of the copper(II) complex $Cu(MK\text{-}H)_2 \cdot 2H_2O$ are reported for the anti-inflammatory drug 2-aminomethyl-4(1,1-dimethylethyl)-6-iodophenol (MK-447). The diffuse reflectance spectra and magnetic moments are consistent with a tetragonally distorted pseudo-octahedral environment around the copper(II) ion. The infrared spectra, indicate that MK-447 acts as a chelate monoanionic ligand with coordination involving the phenolate oxygen atom and the nitrogen atom of the aminomethyl group. The copper(II) complex exhibits marked superoxide dismutase activity in the nitroblue tetrazolium assay.

Introduction

Rheumatoid arthritis is an autoimmune disease, characterised by extensive infiltration of activated polymorphonuclear leucocytes and chronic inflammation of the synovial joint space that results in pain, swelling and progressive erosion of the joint. Activated leucocytes respond to an inflammatory stimulus with an increase in oxygen metabolism [1]. Molecular oxygen is reduced initially to the superoxide radical anion $O_2^{\cdot -}$ and hydrogen peroxide H_2O_2, both species being intended for the destruction of ingested microorganisms within the phagocytic vacuole [2]. The intracellular concentration of superoxide radical anions is controlled by the superoxide dismutases, a group of enzymes that are able to disproportionate superoxide anions [3].

$$2O_2^{\cdot -} + 2H^+ - O_2 + H_2O_2$$

Superoxide anions can escape from the surface of the leucocyte and if not removed can be reduced further to more potent oxygen species such as the hydroxyl radical ·OH [4]. Reactive oxygen species of this type can damage essential macromolecules and have been implicated in the general tissue damage observed in inflammatory disorders [5].

The accumulation of superoxide anions in the synovial fluid as a result of reduced extracellular superoxide dismutase activity has been suggested as a contributing factor in arthritic and other inflammatory diseases [6]. Molecules capable of scavenging extracellular superoxide anions may prevent the formation of more reactive oxygen species and subsequent tissue damage. Bovine superoxide dismutase contains a copper(II) ion as the active central metal ion but the metalloprotein has limited anti-inflammatory activity on intravenous administration due to its rapid clearance from extracellular fluid [7]. Oxygen radical production is inhibited not just by specific metalloproteins but also by free hydrated copper(II) ions and by a number of low molecular mass copper(II) complexes [8]. Copper complexes of non-steroidal anti-inflammatory drugs have been reported to be more effective anti-inflammatory agents and less ulcerogenic than the parent anti-inflammatory drug [9].

The majority of clinically useful non-steroidal anti-inflammatory drugs are carboxylic or enolic acids [10]. The phenolic compound, 2-aminomethyl-4(1,1-dimethylethyl)-6-iodophenol (MK-447; abbreviation MK) exhibits both anti-inflammatory and analgesic activity in animal models and is a recognised oxygen free radical scavenger [11]. In this paper, we report the preparation and properties of the complex formed between MK-447 and the copper(II) ion and compare the superoxide scavenging activity of this copper compound with the neutral and protonated drug.

Experimental

Copper(II) chloride dihydrate $CuCl_2 \cdot 2H_2O$ was of Analar reagent grade. $MK\text{-}447H^+Cl^-$ was supplied by

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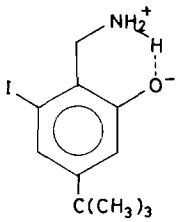
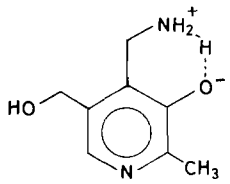


MK 447



Pyridoxamine

Merck, Sharp and Dohme Ltd and used without further purification. Neutral MK-447 was prepared by precipitation on addition of 0.1 M sodium hydroxide to an aqueous solution of the hydrochloride.

Preparation of $Cu(MK\text{-}H)_2 \cdot 2H_2O$

$MK\text{-}447H^+Cl^-$ (1 g; 1 mol. equivalent) dissolved in distilled water (20 cm^3) was added slowly with stirring to a cold solution of copper(II) chloride (0.25 g, 0.5 mol equivalent) in distilled water (20 cm^3). The pH of the resulting mixture was adjusted to 12 by the slow addition of 0.1 M sodium hydroxide solution. The precipitated complex was filtered off under vacuum, washed with cold distilled water and dried at 105 °C.

Microanalysis

Carbon, hydrogen and nitrogen analysis was performed in the microanalytical laboratory of the University College of North Wales, Bangor. Calc. for $Cu(MK\text{-}H)_2 \cdot 2H_2O$: C, 37.31; H, 4.81; N, 3.96. Found: C, 37.68; H, 4.63; N, 3.62%.

Physical Measurements

The diffuse reflectance spectra were determined on a Beckmann DK-2A spectrophotometer fitted with a standard reflectance attachment. Infrared spectra (4000–200 cm^{-1}) were recorded on a Perkin-Elmer 580 spectrometer as potassium bromide discs. The room temperature magnetic susceptibility measurement was made on a powdered sample of the complex using a Johnson Matthey Magnetic Susceptibility MSB1. The superoxide dismutase activity was assayed by the nitroblue tetrazolium method as reported previously [12].

Results and Discussion

The microanalytical results show that the complex is obtained as a dihydrate from aqueous solution with the MK-447 molecule present as a mono-anionic ligand.

Infrared Spectra

The assignment of the various bands (Table 1), without being rigorous, has been made by a direct comparison between the spectrum (4000–400 cm^{-1}) of coordinated $MK\text{-}447^-$, the free base MK-447 and the protonated base $MK\text{-}447H^+$. Neutral MK-447 shows structural similarities to pyridoxamine in which the zwitterionic form results from photon transfer between the acidic phenol group and the basic amine group [13]. The IR spectrum of the protonated molecule $MK\text{-}447H^+$ was run to simplify the assignment of infrared bands and to examine the possibility of zwitterion formation in the free base.

MK-447 possesses two potential donor sites; (i) a phenolate oxygen and (ii) a primary amine nitrogen. These donor sites occupy adjacent positions on the ligand and therefore six-membered chelate ring formation is possible on complexing.

The IR spectra of the protonated and neutral ligand are characterised by broad intense absorption bands above 2000 cm^{-1} with other absorption bands superimposed on this main absorption. The protonated ligand $MK\text{-}447H^+$ shows a broad absorption band at 3400 cm^{-1} that forms part of the general absorption in this region of the spectrum and is assigned to the O–H stretch of the phenol group [14]. The broadness of this and the out-of-plane O–H deformation band at 1312 cm^{-1} in the protonated drug spectrum is indicative of a hydrogen bonded $-NH_3^+$ group. Strong $O\cdots H\text{–}NH_2^+$ intramolecular hydrogen bonding has been observed previously in the infrared spectra of protonated amino acids [15]. The absence of both the O–H stretch and deformation bands from the IR spectra of the neutral drug and the metal complex suggests that the drug is attached to the copper(II) centre as an anionic ligand via the phenolate oxygen and that the neutral drug molecule exists in a zwitterionic form. The presence of the $-NH_3^+$ asymmetric stretching (3005 ± 5 cm^{-1}) and $-NH_3^+$ deformation (1479 ±

TABLE 1. IR Absorption Bands (cm^{-1})

	O–H stretching	O–H deformation	NH_3^+ asymmetric stretching	NH_3^+ deformation	C–N stretching	$-NH_2$ stretching
$MK\text{-}447H^+$	3400m,br	1312s,br	3000s,sh	1478s	1026m,br	
MK-447			3010m,sh	1480m,sh	1029m,br	
$Cu(MK\text{-}H)^2 \cdot 2H_2O$					1018m	3310m

1 cm^{-1}) bands further supports the suggestion that the neutral drug molecule exists as a zwitterion. The band observed at 1027 ± 2 cm^{-1} in the free and protonated ligand and assigned to the C–N stretch of the primary amine group, is lowered to 1018 cm^{-1} in the copper(II) complex [16]. The medium intensity band observed at 3310 cm^{-1} in the copper(II) complex is assigned to the NH_2 stretching band of the coordinated primary amine group [17].

Three C–H stretching vibrations can be assigned as part of the low frequency absorption band. The asymmetric and symmetric C–H stretching vibrations of the –CH group are recorded at 2908 ± 2 and 2868 ± 2 cm^{-1} respectively while the C–H stretch of the $-CH_3$ group occurs as a very strong absorption at 2960 cm^{-1} [15].

A comparison of the IR spectra suggests that the neutral drug molecule exists as a zwitterion and that in the copper(II) complex MK-447 acts as a mono-anionic ligand bonded through the phenolate oxygen atom. It is likely that a second metal ligand bond is formed through the amine nitrogen atom of the aminomethyl group resulting in a six-membered chelate ring.

Electronic Properties

The diffuse reflectance spectrum of MK-447 exhibits a number of low intensity absorption bands that are considered to be infrared overtones. The reflectance spectrum of $Cu(MK\text{-}H)_2 \cdot 2H_2O$ consists of these ligand bands slightly intensified and in addition a broad, low intensity shoulder centred at 17857 cm^{-1} that forms part of the charge transfer band. The position, band shape and intensity suggest a distorted octahedral environment around the copper(II) ion. The effect of a tetragonal distortion on an octahedral copper(II) ion is to split the ^2Eg and $^2T_{2g}$ states so that as the energy of the $^2A_1$ state increases, a situation may arise in which this state is sufficiently close to the ^2E and $^2B_2$ states for the three transitions not to be resolved in the spectrum [18]. In the absence of any other bands in the $Cu(MK\text{-}H)_2 \cdot 2H_2O$ spectrum, it is concluded that all three transitions lie within the single broad envelope and this assignment is in agreement with the general observation that copper(II) d–d transitions are normally close in energy [19]. The magnetic moment of 1.88 BM falls within the range observed for mononuclear complexes having no appreciable interaction between the copper(II) centres.

Pyridoxamine (PM) is a member of the vitamin B_6 family of compounds that has structural similarities to MK-447. The vitamin B_6–metal complexes have been extensively studied as enzymic models in the metabolism of amino acids [20]. The crystal structure of the pyridoxamine complex $Cu(PM\text{-}H)_2 \cdot 2H_2O$ shows the copper(II) ion in a tetragonally distorted octahedral environment with the pyridoxamine anions chelated through the 4-(aminomethyl) and phenolate groups. The equatorial plane around each copper(II) ion is formed by two amino nitrogen atoms and two phenolate oxygen atoms with the axial positions occupied by oxygen atoms from hydroxymethyl groups [21]. The structure of $Cu(MK\text{-}H)_2 \cdot 2H_2O$ is therefore most likely to be *trans*-octahedral D_{4h} with two MK-447 anionic ligands coordinated in the *xy* plane via the N atom of the 2-(aminomethyl) group and the O atom of the phenolate group with two water molecules completing the six-coordinate structure.

Superoxide Assay

The superoxide dismutase activity of the free drug MK-447, the protonated drug $MK\text{-}447H^+$ and the copper(II) complex $Cu(MK\text{-}H)_2 \cdot 2H_2O$ were assayed by their ability to inhibit the reduction of nitroblue tetrazolium as described previously [12]. MK-447 is a known oxygen free radical scavenger [20]. The scavenging data reported here (Table 2) indicates that the $Cu(MK\text{-}H)_2 \cdot 2H_2O$ complex exhibits a greatly increased superoxide dismutase activity compared with both the parent drug MK-447 and the protonated drug $MK\text{-}447H^+$. The results are consistent with earlier observations that superoxide radical anion scavenging is not restricted to the metalloprotein superoxide dismutase but exhibited by a number of low molecular mass copper(II) complexes.

TABLE 2. Superoxide Assay

Additive	% Absorbance increase at 560 nm compared with blank
$MK\text{-}447H^+$	72
MK-447	73
$Cu(MK\text{-}H)_2 \cdot 2H_2O$	2

The mechanism proposed for the dismutisation of superoxide anions by both superoxide dismutase and metal complexes is thought to involve redox cycling of copper(II) ions:

$$Cu^{2+} + O_2^{\cdot -} \rightleftharpoons Cu^+ + O_2$$

$$Cu^+ + O_2^{\cdot -} + 2H^+ \rightleftharpoons Cu^{2+} + H_2O_2$$

The $O_2^{\cdot -}$ binds directly to the metal centre by rapid exchange of coordinated water followed by electron transfer between the copper(II) centre and the oxygen radical anion. We consider that in the tetragonally distorted octahedral complex $Cu(MK\text{-}H)_2 \cdot 2H_2O$ the *trans* weakly-bonded water molecules are readily substituted by $O_2^{\cdot -}$.

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